(B) *Efficiency of the column.* From the number of theoretical plates (n) calculated as described in § 436.216(c)(2) of this chapter, calculate the reduced plate height (h_r) as follows:

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:

L=Length of the column in centimeters;
n=Number of theoretical plates; and
d_p=Average diameter of the particles in the analytical column packing in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 15.

(C) *Resolution factor.* The resolution factor (R) between the peak for clindamycin phosphate and the peak for clindamycin (hydrochloride) in the chromatogram of the resolution test solution is satisfactory if it is not less than 6.0.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation ($S_R$in percent) of 5 replicate injections of the working standard solution (prepared as directed in paragraph (b)(1)(ii)(A) of this section is satisfactory if it is not more than 2.5 percent.

If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations.* Calculate the clindamycin content as follows:

$$\text{Milligrams of clindamycin per milliliter} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the clindamycin phosphate peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);
A_s=Area of the clindamycin phosphate peak in the chromatogram of the clindamycin phosphate working standard;
P_s=Clindamycin activity in the clindamycin phosphate working standard solution in micrograms per milliliter; and
d=Dilution factor of the sample.

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted lotion.

(3) *Identity.* The high-performance liquid chromatogram of the sample determined in paragraph (b)(1) of this section compares qualitatively to that of the clindamycin phosphate working standard.

[54 FR 40655, Oct. 3, 1989]

§ 453.522d Clindamycin phosphate vaginal cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clindamycin phosphate vaginal cream contains clindamycin phosphate in a suitable and harmless cream vehicle. Each gram contains clindamycin phosphate equivalent to 20 milligrams of clindamycin activity. Its clindamycin content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of clindamycin that it is represented to contain. Its pH is not less than 3.0 and not more than 6.0. It passes the identity test. The clindamycin phosphate used conforms to the standards prescribed by § 453.22(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The clindamycin phosphate used in making the batch for clindamycin content, microbiological activity, moisture, pH, crystallinity, and identity.

(B) The batch for clindamycin content, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The clindamycin phosphate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(B) The batch: a minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Clindamycin content (high performance liquid chromatography assay).* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 210 nanometers, a 25-centimeter long x 4.6 millimeter ID column packed with microparticulate (5 to 10 micrometers in diameter) reverse

phase octylsilane hydrocarbon bonded silica packing material, a flow rate of 1.0 milliliter per minute, and a known injection volume of 20 microliters. The retention time of clindamycin phosphate, and clindamycin are approximately 6 and 9 minutes, respectively. Reagents, working standards and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *0.1M Potassium phosphate monobasic buffer.* Dissolve 13.61 grams of potassium phosphate monobasic in 775 milliliters of water. Adjust the pH to 2.5 with phosphoric acid. Further dilute with water to a volume of 1,000 milliliters.

(B) *Mobile phase.* Mix 225 milliliters of acetonitrile and 775 milliliters of 0.1*M* potassium phosphate, pH 2.5 buffer (225:775). Filter through a suitable filter capable of removing particulate matter greater than 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A) *Working standard solution.* Dissolve an accurately weighed portion of the clindamycin phosphate working standard in sufficient mobile phase (prepared as directed in paragraph (b)(1)(i)(B) of this section) to obtain a solution containing 200 micrograms of clindamycin activity per milliliter.

(B) *Sample solutions.* Accurately weigh and transfer approximately 1.0 gram of the sample into a 125-milliliter Erlenmeyer flask. Add 100.0 milliliters of mobile phase (prepared as directed in paragraph (b)(1)(i)(B) of this section), accurately measured, and 8 to 10 glass beads (4 to 5 millimeters). Close the flask securely using a plastic stopper and shake vigorously by mechanical means for 1 hour at 50 °C. Cool in an ice bath for approximately 20 minutes. Centrifuge a portion of the mixture. Use the lower cloudy solution for chromatographic analysis. Filter a few milliliters of the centrifuged solution through an appropriate 2 micron filter.

(C) *Resolution test solution.* Place 15 milligrams each of clindamycin phosphate and clindamycin hydrochloride in a 25-milliliter volumetric flask and dissolve and dilute to volume with mobile phase and mix well. Use this solution to determine the resolution factor.

(iii) *System suitability requirements*—(A) *Asymmetry factor.* Calculate the asymmetry factor (A_S), measured at a point 5 percent of the peak height from the baseline as follows:

$$A_s = \frac{a+b}{2a}$$

where:

a = Horizontal distance from point of ascent to point of maximum peak height; and

b = Horizontal distance from point of maximum peak height to point of descent.

The asymmetry factor (A_s) is satisfactory if it is not less than 1.0 and not more than 1.3.

(B) *Efficiency of the column.* From the number of theoretical plates (*n*) calculated as described in §436.216(c)(2) of this chapter, calculate the reduced plate height (h_r) as follows;

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:

L = Length of the column in centimeters;

n = Number of theoretical plates; and

d_p = Average diameter of the particles in the analytical column packing in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 15.

(C) *Resolution factor.* The resolution factor (*R*) between the peak for clindamycin phosphate and the peak for clindamycin (hydrochloride) in the chromatogram of the resolution test solution is satisfactory if it is not less than 6.0.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent) of 5 replicate injections of the working standard solution is satisfactory if it is not more than 2.5 percent. If the system suitability parameters have been met, then proceed as described in §436.216(b) of this chapter.

(iv) *Calculation.* Calculate the clindamycin content as follows:

$$\text{Milligrams of clindamycin per gram} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u = Area of the clindamycin phosphate peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s = Area of the clindamycin phosphate peak in the chromatogram of the clindamycin phosphate working standard;

P_s = Clindamycin activity in the clindamycin phosphate working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample.

(2) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted cream.

(3) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the clindamycin phosphate working standard.

[60 FR 49508, Sept. 26, 1995]

PART 455—CERTAIN OTHER ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.

Subpart B—Oral Dosage Forms

Subpart C—Injectable Dosage Forms

Subpart D—Ophthalmic Dosage Forms

Subpart E—Otic Dosage Forms

Subpart F—Dermatologic Dosage Forms

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